MoP152 Catheter-related *M. fortuitum* infection associated with continuous ambulatory peritoneal dialysis (CADP)

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Description of the case: A 65-year-old man with renal failure due to focal glomerulonephritis.

After a Tenckhoff catheter was implanted, was submitted to CADP. Five months later, the patient developed a purulent discharge at the peritoneal catheter exit site. Purulent material was sent to microbiology for Gram stain and routine cultures, and *S. epidermidis* was isolated; and the patient was treated with topical mupirocin. Two weeks later, peritoneal dialysis effluent remained clear, but the patient continued to have whitish purulent drainage from the exit site. A new culture of the drainage was performed. Gram stain of this sample showed atypical rods stained uncharacteristically, which motivated to make a Ziehl-Neelsen stain and to prolong the incubation time. After 5 days, pin-point colonies similar to Corynebacterium species were isolated. Gram stain of these colonies showed atypical gram-positive rods with nonstaining areas; Ziehl-Neelsen stain showed acid-fast bacilli. The organism was identified as *M. fortuitum*. Minium inhibitory concentrations were determined. Treatment with ciprofloxacin for 5 weeks and azithromycin for 2 weeks was administered orally. Three months later, purulent exit side infection continue with cultures positives for *M. fortuitum* and the therapy was changed to intraperitoneal amikacin and cefoxitin for two weeks. Cefoxitin therapy was maintained for 4 weeks. A new culture was negative. The patient was continued on CADP during the treatment period and the peritoneal catheter was not removed. There is no evidence of infection at this time.

Conclusion: The source of this infection was not determined. The repeated isolation of *M. fortuitum* from the catheter exit site, in the absence of other organisms suggests that the *M. fortuitum* was responsible for the infection. The appearance of these organisms in Gram stain is atypical, and they may be overlooked or confused with debris or diphtheroids. Ziehl-Neelsen stain and to prolong the incubation time are necessary for the recovery of these organisms.

MoP153 Intramedullary tuberculoma confirmed by PCR

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Objective: Central nervous system tuberculosis is rare, affecting only 0.5 to 2% of all patients with systemic tuberculosis. Intramedullary tuberculomas are extremely rare, with approximately 150 cases reported id the world literature. The objective of this report is to describe a case of intramedullary tuberculoma and its confirmation by PCR.

A 46 year old female presented with numbness and weakness of the left lower extremity for one year, associated with urinary frequency and urgency. Numbness had progressed to the rib cage. She suffered from night sweats and subjective fever for one year. She reported a family history of tuberculosis, but her chest radiograph was normal, and her PPD was negative. She presented lower extremity weakness, 4/5 motor strength, decreased sensory to touch (up to rib cage), positive left Babinsky sign, and no clonus. The magnetic resonance imaging (MRI) of her spine revealed a syrinx which enhanced at T9–T11 level, indicative of spinal cord lesion.

Methods and: Results: The spinal cord lesion was surgically resected. The pathology analysis revealed granulomatous myelitis and acid-fast bacilli were observed after ZiehlNeelsen stain. To confirm the histologic findings thin sections of paraffin-embedded tissue were used for DNA extraction using a Generation DNA Purification System (Gentra Systems, Minneapolis, MN). Successful DNA isolation was confirmed by amplification of beta-globin as an in-house keeping gene. A 356 bp fragment of Mycobacterium tuberculosis DNA was amplified by an IS6110-nested PCR (Montenegro-James *et al.* 1998). The patient was started on 4 drug therapy, isoniazid, rifampin, ethambutol, and pydzanamide and steroids. The patient's sensory, motor deficits, and urinary function improved two months after treatment. A 3 month post-surgery MRI showed thoracic cord atrophy. Patient has completed 24 months of antituberculous therapy.

Conclusions: Intramedullary tuberculoma is an uncommon presentation of tuberculosis that occurs as a subacute spinal cord compression syndrome (weakness, paresthesias, bowel and bladder dysfunction) that may not be associated with pulmonary TB. Treatment included surgery and chemotherapy. Histopathological diagnosis demonstrated the presence of acid-fast bacilli and granuloma. In absence of microbiological confirmation, demonstration of MTB DNA by PCR is desirable. Intramedullary TB should be considered in the differential diagnosis of patients with suspected spinal cord compression. PCR can be used for rapid detection of MTB DNA.

MoP154 A one year prospective study using molecular strain typing for the evaluation of tuberculosis transmission in paris suburb: Preliminary results

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Objectives: To define the risk-factors associated with transmission of tuberculosis using a restriction fragment length polymorphism (RFLP) analysis among patients with culture positive for *Mycobacterium tuberculosis (M. tb)*.

Methods: A prospective population-based study was conducted between May 1997 and 1998 in Val de Marne (1.25 millions inhabitants). IS6110 RFLP was performed on the *M. tb* isolates. Social and behavioral data, clinical data and antituberculous drug resistance were collected.

Results: 222 cases will be presented. To date 129 cases have been interviewed and RFLP completed. 14% of the strains were resistant to at least one antituberculous drug, 80% of HIV status were available of whom 13% were positive. Furthermore 65% were born outside of France, 32% had an annual income below the official poverty line and 15% lived in emergency accomodation of whom 6% had no fixed abode. Among the 129 cases 39 (30%) were potentially linked. They are grouped into 16 clusters: 12 clusters contained 2 cases, 4 clusters from 3 to 5 cases.

Conclusion: Preliminary results show that among the 129 cases with RFLP analysis available 30% are potentially linked.

Topic 5 – In vitro activity of antimicrobial agents

P:5/1 – Aminoglycosides

WeP34 Eect of sub-inhibitory concentrations of gentamicin on haemagglutination of uropathogenic Escherichia coli

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Urinary tract infections are a leading cause of morbidity and health care expenditures in persons of all ages, and Escherichia colt is the most common cause of urinary tract infections. At some point during their lives, at least 12% of men and 10 to 20% of women experience on acute symptomatic bacteriuria.

Bacterial adhesion to the mucosal surface of the urinary tract is considered to be an important first step in the bacteria-host interactions which give rise to infection. The fimbriae responsible for this attachment aid the migration of pathogenic organisms, originating from the faeces, up into the urinary tract to the bladder and on to the kidneys. It has been known for some time that antibiotics at sub-inhibitory concentrations, can inhibit the production of bacterial virulence factors in vitro.

In this study, gentamicin were tested for their effect on haemagglutination of the mannose-resistanthaemagglutination positive Escherichia coli, isolated from patients with urinary tract Infections. Minimum inhibitory concentrations of gentamicin for these strains were determined in muller-hinton broth, and the effect of each antimicrobial, at the quarter MIC values, on the haemagglutination of the strains was then determined.

WeP35 Detection of gram-negative resistance to aminoglycosides with PhoenixTM 100 System

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Objectives: To evaluate the performance of the PhoenixTM 100 Automated Microbiology System (currently under development) in detecting resistance to aminoglycosides in gram-negative bacilli.
Methods: A total of 210 isolates, including fresh clinical isolates (100 isolates) and a challenge set (110 strains) with various known resistance mechanisms, were tested in parallel in a NCCLS recommended Standard Broth Microdilution (SBM) procedure and in the Phoenix System. Doubling dilutions of six aminoglycosides (Amikacin, Gentamicin, Isepamycin, Kanamycin, Netilmicin and Tobramycin) were prepared according to the NCCLS guidelines. Reference SBM panels were manually read after 16–20 h of incubation at 35°C in ambient air. Phoenix panels were tested with an inoculum concentration of 5 × 105 cfu/ml, incubated and kinetically read by the instrument every 20-min.
Results: Essential Agreement between the two procedures for the antimicrobial agents tested ranged from 91 to 97%, with no very major error (VME) or major error, except Isepamycin and Tobramycin (1 strain with VME). The average time to results was 6 to 9 h for most enteric isolates and 9 to 13 h for other gram-negative bacilli.
Conclusions: The results of this evaluation indicate that the Phoenix system can provide a rapid detection of aminoglycoside resistance among gram-negative bacilli for clinical laboratories.

WeP36 Novel streptomycin resistant mutants of *Salmonella typhimurium* isolated in an *In Vitro* kinetic model

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Objectives: *S. typhimurium* LT2 was used for selection of streptomycin resistant sub-populations in an *in vitro* kinetic model. Other models with static concentrations does not reflect the potential selective properties of fluctuating levels achieved during treatment.
Methods: Bacteria were exposed to two doses of streptomycin at 10× respective 500 × MIC in an *in vitro* kinetic model. The elimination rate was set to achieve MIC at 12 h for both dosing regimens and the second dose was given at 12 h. Samples were withdrawn at different times during 24 h and cultured on plates with 0, 2, 50 and 500 × MIC of streptomycin. The selected mutants were further investigated by PFGE, PCR and Southern hybridisation to identify the mutation sites.
Results: With two exposures of 500 × MIC maximal killing effect was obtained after 24 h and no mutants were isolated. With 10 × MIC there was no significant killing effect of the bacteria and regrowth occurred after 6 h. Ten resistant clones with MIC values between 32 and > 1024 mg/l were chosen for further studies. One high-level resistant isolate contained a previously described point mutation in the *rpsL* gene. The remaining isolates had mutations at novel sites identified in the *aadA*, *unc* and *citA* genes.
Conclusions: An initial dose of 10 × MIC followed by decreasing concentrations selected for other types of streptomycin resistant mutants as compared to previous studies using agar plate selection. The emergence of these mutants was prevented by a higher dose.

WeP37 Resistance surveillance of greek *Haemophilus influenzae* (*H.I.*) strains isolated from healthy carriers in 1997–1998

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Resistance surveillance of H.I. in Greece, derived mostly from specimens of patients with chronic bronchitis and performed in 1988, revealed resistance rates to Ampicillin 28.3%, to Cefaclor 2.7%, to Tetracycline 10.6%, and to Trimethoprim/Sulfamethoxazole (TMP-SMZ) 5.3%. To extend our surveillance follow-up, a study was organized in the winter 1998. Samples from 608 healthy individuals were obtained with naso-pharyngeal swabs: 189 from adults and 419 from children 3–7 years old. The rate of isolation of *H.I.* was 5.8% for the adults and 36.5% for the children (11 and 153 isolates respectively). Socioeconomic classes differences were irrelevant to the rate of nasopharyngeal carriage. 159 isolates were tested and MICs (μg/ml) were performed by a microdilution technique using HTM broth. Resistance to Ampicillin (MIC > 2) was 13.2%, to Cefaclor (MIC > 16) 0.6%, to Tetracycline (MIC > 4) 4.4%, to TMP/SMZ (MIC > 1/19) 13.83%, to Clarithromycin (MIC > 16) 2.3% while MICs90 were as follows: Ampicillin < 2 (MIC50 < 12); Amoxicillin/Clav < 2/1; Ampicillin/Sulb < 1/0.5; Cefaclor < 4; Cefuroxime < 1; Cefixime < 0.12; Ceftriaxone < 0.03; Cefepime < 0.12; Imipenem < 0.5; Rifampin < 0.5; Tetracyclin < 0.5; Chloramphenicol < 0.25; TMP/SMZ < 2/38; Ciprofloxacin < 0.015; Trovafloxacin < 0.03; Grepafloxacin < 0.03; Sparfloxacin < 0.03; Clarithromycin < 8. It is concluded that resistance rates of H.I. to classical antimicrobial agents is decreasing, a fact probably attributed to the increased use of newer macrolides in the community.

WeP38 Which is the appropriate technique to asses the susceptibility of Staphylococcus aureus in clinical laboratory? (Aminoglycosides, MLSB group and Fluoroquinolones)

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Objectives: To asses the appropriate technique to test the MLSB, aminoglycosides and quinolones resistance of staphylococci.
Methods: The susceptibility of 60 Staphylococcus aureus (SA) and 50 coagulase-negative staphylococci (CNS) strains belonging to 8 species (biochemical identification by API-system Merieux) was tested in Mueller-Hinton agar by disk diffusion, agar dilution and E-test against Oxoid standard disks and the agar dilution (mg/L) for gentamicin (4 and 8), kanamycin (6 and 25), amikacin (16 and 32), netilmicin (12 and 32), ciprofloxacin (1 and 4), clarithromycin (2 and 8), lincomycin (1 and 8) and erythromycin (15 μg disk).
Results: From 25/60 SA and 31/50 CNS Oxa 4 mg/L resistant isolates there was as resistant strains: 22/25 SA and 27/31 CNS to aminoglycosides (almost all with Gen Kan Amk phenotype), 20/25 SA and 15/31 CNS to ciprofloxacin and 19/25 SA (14 inducible) and 23/32 CNS (7 inducible) to MLSB. For oxacillin susceptible strains the figures was respectively for aminoglycosides 11/35 SA (phenotype Kan) and 7/19 CNS, for quinolones 4/35 SA and 2/19 CNS and for MLSB group 7/35 SA and 11/19 CNS.
Conclusion: With a good agreement between techniques for aminoglycosides and quiolones it is needed to test reducibility for MLSB.

P:5/2 – Betalactam agents

WeP39 Conjugative resistance to tazobactam plus piperacillin among extended-spectrum beta-lactamase producing nosocomial *Klebsiella pneumoniae*

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Objective: To demonstrate the genetic location and enzymatic characteristics of piperacillin-tazobactam resistance in extended-spectrum beta-lactamase (ESBL) producing nosocomial *Klebsiella* isolates.
Methods: Transconjugation experiments were studied on agar media and the recipients were selected on Mueller-Hinton agars supplemented by rifampicin plus either ceftazidime or piperacillin. Isoelectric-focusing were achieved on acrylamide/bis-acrylamide gels having ampholytes with pI range of 3 to 10. Minimal inhibitor concentrations of antibiotics were determined by agar dilution method. TEM genes from the isolates were amplified by polymerase chain reaction.
Results: A total of 30 ESBL producing *Klebsiella pneumoniae* from different hospitals were studied. From these, we obtained 7 transconjugates resistant to piperacillin-tazobactam, 23 to ceftazidime plus tazobactam (also to piperacillin-tazobactam) and 10 to only ceftazidime. Piperacillin-tazobactam resistant isolates were producing a single enzyme with pI point of 5.4. All of these were *bla*TEM positive and the sequence of a single amplification product was identical to TEM-1.
Conclusion: The ubiquitous classical beta-lactamase TEM-1 is responsible from the conjugative resistance of ESBL producing *Klebsiella pneumoniae* to tazobactam in Turkish hospitals.